

Table 5

Food preferences of dorid nudibranchs in the laboratory.
The relative amount of any sponge eaten by one nudibranch was estimated by the proportion of the characteristic spicule types in a well-mixed sample of the feces of that animal (see text for further details).

Dorid species	Sample size	Caecate or acaecate	Percent appearing in feces			
			<i>Halichondria panicea</i> non-reticulate	<i>Myxilla incrustans</i> semi-reticulate	<i>Haliclona permollis</i> isodictyal	<i>Mycale adhaerens</i> highly reticulate
<i>Archidoris montereyensis</i>	3	C	83	17	0	0
<i>Archidoris odhneri</i>	3	C	53	30	0	17
<i>Anisodoris nobilis</i>	3	A	2	20	5	73
random samples of mosaic	6		27	25	23	25

REST, 1953), or it does not (FOURNIER, 1969; BLOOM, 1974). Unfortunately, the digestive morphology of dorids is rarely mentioned in the taxonomic literature and the presence or absence of a caecum must be inferred from other dorids within a given subfamily.

Sponge skeletal morphology is also quite diverse, but tends to be similar within a given order. The order Halichondrida is characterized by spicules and spongin "intermingled without definite localization" (HYMAN, 1940). Bundled megascleres characterize the order Hadromerida while an isodictyal pattern (a pattern in which a 3-dimensional lattice is formed by spicules interconnected at their tips by spongin) characterizes the order Haplosclerida. The large order Poecilosclerida has a variety of skeletal types but is, in general, characterized by a reticulate network of interconnected spicules and spongin (HYMAN, *op. cit.*). Sponges, then, can be arranged to form a discontinuous resource gradient with regard to increasing difficulty of fragmentation. In other words, the first sponges would be the non-reticulated sponges (Halichondrida), followed by the bundled sponges (Hadromerida), then the semi-reticulated sponges (Haplosclerida) and ending with the highly reticulated sponges (Poecilosclerida). There are exceptions to these generalities and the actual descriptions of the skeletons of sponges which appear in dorid diets are given in Table 1 and their ranking is presented in Table 3.

The presence of a caecum appears to be a critical factor in dorid digestive strategies. A dorid with a caecum can handle large quantities of large and usually sharply-pointed spicules released by digestion of an unorganized or non-reticulated sponge. However, modifications of the

radula and the intestine to handle non-reticulated sponge tissue and fecal-spicule ropes respectively appear to exact an energetic disadvantage when feeding on a more-reticulated sponge (the data supporting these generalities will be presented in a forthcoming paper).

Conversely, the absence of the caecum, paired with a more robust radula and a more muscular intestine, appear to be adaptations to a more-reticulated prey. Utilizing caecal and radular characteristics, the prediction is that animals with a caecum should preferentially consume non-reticulated sponges while animals without a caecum should preferentially consume reticulated sponge prey. Animals with more robust radulae, *i. e.*, fewer but larger and more strongly-hooked teeth, should preferentially consume more-reticulated sponges than animals sharing the same caecal characteristics but having less robust radulae. Due to lack of information on the size of radulae relative to the size of the animals, the only consistent measure of radular robustness readily available is the degree of "hook" or curvature of the radular teeth (Figure 1).

These predictions can be tested by regarding the data presented in Table 3 as points plotted on a Cartesian coordinate system and statistically analyzing the point distribution for randomness and correlation between the axes. The horizontal axis is the discontinuous resource gradient of sponges mentioned earlier, with non-reticulated sponges on the left. The vertical, or dorid, axis is arranged with all caecate animals as a group placed above all acaecate animals. Within these 2 categories, the subfamilies and the species within the subfamilies are arranged by mean radular tooth-curvature with the degree of hook increasing from top to bottom.

Given the arrangement of these axes, the prediction made above would imply a diagonal cluster from upper left (caecate, non-reticulated) to lower right (acaecate, reticulated). Visually, there does appear to be such a cluster (Table 3).

These data were analyzed statistically by regarding the table as a contingency table and testing for randomness. The results of such testing are presented in Table 4. When Table 3 is regarded as a 2×2 contingency table (caecate vs. acaecate; non-reticulated vs. reticulated), the chi-square statistic is sufficiently large to allow rejection of the null hypothesis of a random point distribution at the 0.025 level.

Further subdivision of the sponge axis results in an even more significant rejection ($p < 0.001$). This increase in the confidence that there is a relation between the axes may well be due to the addition of radular hook information. The correlation of the 2 axes is 0.45 (Contingency Coefficient) and the correlation is significant at the 0.001 level (SIEGEL, 1956).

Laboratory food preferences demonstrate the same pattern. The experimental design was such that the dorids were exposed to equal quantities of 4 sponges of widely varying skeletal complexity by placing the animals on a well-mixed sponge mosaic. If the ratio of characteristic spicule type in the feces for the 4 sponges was approximately equal to the ratio of those spicules in the control samples, the dorid producing the feces would have treated the mosaic in a generalized manner. If the ratio in the feces differed markedly from the ratio in the controls, the animal preferentially selected only certain grain-types in the mosaic. As shown in Table 5, caecate animals preferentially consumed non-reticulated sponges while acaecate animals preferentially consumed reticulated sponges.

The demonstration that there is a correlation between sponge and dorid morphologies may help to explain some of the puzzling variations in dorid morphology and is proof that there are specializations within the category of sponge-rasping dorid nudibranchs.

SUMMARY

1. Dorid digestive morphology is reviewed and the hypothesis that there are specializations within the category of sponge-rasping dorid nudibranchs as shown by a correlation of dorid morphology to sponge skeletal morphology is advanced.
2. Information on dorid diets is collected from a large number of fecal samples of 6 species of dorids found in the San Juan Archipelago, Washington, and from the

literature, and is summarized to allow testing of the hypothesis.

3. There is a statistically significant correlation of dorid-to-sponge morphologies as shown by an analysis of dorid diets in nature.
4. Laboratory feeding-preference experiments support the conclusions reached through correlative means.

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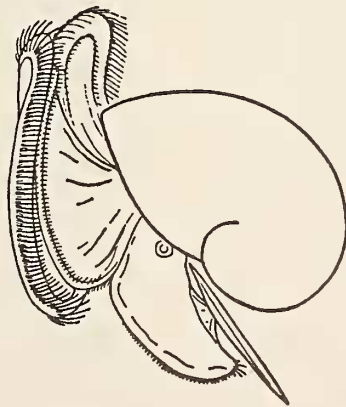
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A Preliminary Study of *Conus* Venom Protein

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(2 Plates)

INTRODUCTION

ALTHOUGH THE TOXICITY of venoms of the genus *Conus* has been known since a report by RUMPHIUS in 1705 (VAN BENTHEM-JUTTING, 1959), these venoms have been studied only intermittently since. Biochemical studies have not been carried out to any great extent; the last major study on the biochemistry of the venom of *Conus* was carried out by KOHN, SAUNDERS & WIENER in 1960.

In this communication, we summarize some preliminary studies on the protein of these venoms. It will be demonstrated that the venoms have a high protein concentration, and evidence will be presented that the proteins are responsible for toxicity. In addition, gel electrophoresis analyses indicate that there are only a few major protein species in each venom, with widely varying molecular weights.

METHODS

(1) Materials

Specimens of mollusks belonging to the genus *Conus* were obtained from several sources in the Philippines. Specimens were collected either near the islands of Cebu, Marinduque or in Batangas Province, Luzon Island. In early studies, the mollusks were frozen in dry ice, and stored at -5°C until the specimens were dissected. Extracted venoms were also stored at -5° , usually diluted with distilled water. In most studies to be described, mollusks were kept alive in salt water aquaria for several months, and the venom was extracted only when it was

to be used immediately. Most studies were carried out with *Conus textile* Linnaeus, 1758 and *C. geographus* Linnaeus, 1758. Typical specimens actually used are shown in Figures 1 and 2.

Reagents used for the preparation of polyacrylamide gels for electrophoresis came from Canal Industrial Corporation, Rockville, Maryland. All organic and inorganic chemicals used were reagent grade. Pronase (B grade) was obtained from Calbiochem, San Diego, California.

(2) Venom Extraction

The venoms from the different species of *Conus* were obtained from venom ducts by placing each duct on an ice-cold metal spatula, then cutting out the duct in 2 cm segments, and squeezing out the contents with a pair of forceps. The venom from each specimen was weighed, then suspended either in distilled water or normal saline solution.

(3) Protein Determination

An aliquot of the venom (usually $5\ \mu\text{l}$ of a 20% suspension) was diluted with 0.4 ml of distilled water. Proteins were precipitated from the diluted sample by adding 0.1 ml of 50% trichloroacetic acid to give a final trichloroacetic acid concentration of 10%. The mixtures were allowed to stand over crushed ice for 10 min and centrifuged at 10 000 RPM in an SS-34 Rotor in the refrigerated RC2B Sorvall Centrifuge. The precipitate obtained was dissolved in 0.1 M NaOH prior to protein determination according to the method of LOWRY (1951).

(4) Disc Gel Electrophoresis

Disc gel electrophoresis was carried out in a Canalco Disc Electrophoresis chamber, model 1200, using formu-

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Table 1

Venom samples were prepared by squeezing fresh venom out of the venom ducts, weighing the venom in a tared vial and adding enough 0.15 M sodium chloride to yield a 20% venom solution. The venom (or control solutions, 0.15 M NaCl or a solution containing 50mg/ml of bovine plasma albumin in 0.15 M NaCl) were injected into mice either intraperitoneally or intracisternally. Protein determinations were carried out as described under Methods; the designation "no data" indicates that these were venom samples on which protein determinations were not carried out.

Comparison of the Toxicity of Venoms from Different *Conus* Species

Venom sample injected	Net wt. of mouse, g	Volume injected (μ l)	Total mg protein injected	Observations
A. Intraperitoneal Injections				
0.15 M NaCl	26	500	—	alive, no significant effect
<i>Conus textile</i>	26	500 ¹	no data	alive, drowsy after injection but became very active again; scratched the abdominal area with hind legs
	25	50	2.5	
	30	50	2.5	
Bovine plasma albumin (50mg/ml)	21	50	2.5	found dead after 2 days
	20	50	2.5	alive
<i>Conus aulicus</i>	30	50	1.73	alive, normal and active right after injection
	31	50	0.93	"
	28	50	1.98	found dead on the following day
<i>Conus geographus</i>	23	50	0.53	died after 7 min
	29	50	0.53	died after 10 min
	27	50	0.53	died after 12 min
<i>Conus tulipa</i>	30	50	no data	died after 36 min
	31	50	no data	died after 27 min gasped and jerked
	25	50	no data	died after 45 min
<i>Conus marmoreus</i>	25	50	0.68	alive; no observed effect
B. Intracisternal injection (All mice were chloroformed before injection)				
0.15 M NaCl (control)	24	50	—	controls looked drowsy most probably from CHCl ₃ but recovered after some time
	23	50	—	
	24	50	—	
	21	50	—	no deaths
	16	50	—	
	19	50	—	
Bovine plasma albumin 50 mg/ml	29	50	2.5	alive; practically same behavior as controls
	21	50	2.5	"
	18	50	2.5	"
	30	50	2.5	"
	23	50	2.5	"
	27	50	2.5	found dead the following day
	35	50	2.5	alive
	21	50	2.5	alive
29	50	2.5	alive	
<i>Conus textile</i>	22	50 ¹	no data	labored breathing; paralysis of right arm; tremor, then death after 30 min.
	26	50 ¹	no data	same difficulty with breathing; gasped for air, eyes turned brownish red after 30 min, died after 50 min; no tremors.
	33	50	2.5	died after 5 min; gasped for air.
	28	50	2.5	died after 25 min; tremors before death.
	32	50	2.5	died after 2 min.
	26	50	2.5	died after 7 min, 30 sec.
	20	50	no data	died after 20 min.

Table 1 (continued)

Venom sample injected	Net wt. of mouse, g	Volume injected (μ l)	Total mg protein injected	Observations
<i>Conus aulicus</i>	27	50	1.73	alive; quickened & shallow breathing; recovered after 22 min.
	25	50	0.93	alive; gasped for air; shallow breathing; recovered after 10 min.
	27	50	1.73	alive; weak; labored breathing; recovered after 6 min.
	no data	50	1.98	alive; weak; normal breathing.
	no data	50	1.0	alive; fast shallow breathing; slight trembling; jumpy.
	25	50	0.93	died after 13 min & 30 sec; weak breathing, foaming of mouth before death.
<i>Conus geographus</i>	28	50	0.53	died after 2½ min; no tremors.
	30	50	0.53	died after 3 min.
	29	25	0.26	died after 5 min.

110% venom. All other samples were 20% venom.

lation prescribed by Canalco for RDS Gels. All electrophoreses were done at 3 milliamperes per tube for at least one hour. Protein bands were visualized by staining the gel with amido black, and destaining the background electrolytically with Canalco quick gel destainer.

RESULTS

(1) Biological Assay for Toxicity

In order to study biological effects of *Conus* venoms, a preliminary survey of toxicity of these venoms was carried out with mice. The results of these studies are shown in Table 1. It is clear that of the species examined, *C. geographus* and *C. tulipa* Linnaeus, 1758 are the only two that are toxic when the venom is injected intraperitoneally. *Conus textile*, *C. aulicus* Linnaeus, 1758 and *C. marmoreus* Linnaeus, 1758 did not cause death of mice under

these conditions. However, it was found that if the venoms were injected intracisternally, the *C. textile* also showed a definite toxic effect. These results are in agreement with the previous biological effects demonstrated by WHYTE & ENDEAN (1962), ENDEAN & RUDKIN (1965), and by KOHN *et al.* (1960).

Autopsies of mice which died from intracisternal injections of *Conus textile* venom showed that lung hematomas were present. On the other hand, mice that died after intraperitoneal injections of *C. geographus* venom showed no hematomas; thus, the mechanism of toxicity may be different. There is considerable variation in the onset of death in mice after injection with 2.5 μ g of *C. textile* venom (2 - 25 min); at lower venom levels, *C. geographus* venom consistently caused death in less than 5 min if injected intracisternally. This supports the general notion that *C. geographus* is probably the most dangerous of all the *Conus* species.

Explanation of Figures 1, 2

Figure 1: Two species of *Conus* reported to have caused injury and death in man. *Conus geographus* is a fish-eating species, while *Conus textile* preys on other mollusks. The scale is in centimeters. These are shells of mollusks actually used in this study.

Figure 2: Venom apparatus of *Conus textile*. A, venom bulb; B, venom duct; C, radula sheath; D, pharynx; E, proboscis. The shell of the mollusk from which the venom apparatus is taken is shown for comparison.